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Effect of hyperbaric oxygen on experimental syphilis in the rabbit

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It is generally accepted that *Treponema pallidum* is a strict anaerobe requiring a very low environmental oxygen reduction potential and to which oxygen is highly toxic. On this assumption investigations have been carried out to explore how far pure oxygen under increased pressure might be effective against experimental syphilis in the rabbit.

The inhibitory and toxic effect of hyperbaric oxygen (HBO) on bacterial growth in culture media (anaerobic and aerobic) has been recognized in various experiments (Boerema and Groeneverd, 1970; Chesney, Turney, and Halley, 1928; Hardy, 1969). However, evidence is largely lacking that this inhibitory or lethal effect can be adequately reproduced *in vivo* in humans or in experimental animals because the use of HBO is limited *in vivo* as prolonged exposure and high tension causes poisoning.

Breathing pure oxygen under hyperbaric conditions at approximately 2·5 atmospheres absolute (that usually used in our experiments) results in full chemical saturation of haemoglobin and in a substantial increase in the amount of oxygen in physical solution in the plasma (a rise of about 6 ml./100 ml. blood).

It was reasonable to suppose that this high tension of pure oxygen and in consequence the increased oxygen supply of the tissues in infected animals might have some toxic or other effect on the anaerobic treponemes and alter the natural course of infection.

Methods and material

For hyperbaric oxygenation in our investigations the experimental hyperbaric system (Vickers Limited, London) was used with the maximum working pressure of four atmospheres absolute and the maximum oxygen flow of 30 l./min.

The investigations were carried out, including control tests, on 64 rabbits which were inoculated with the Nichols strain (testicularly 36, intravenously 18, and cutaneously 10). Of the inoculated rabbits 29 were lost, mostly during or shortly after the exposures to HBO, particularly if they had been exposed longer to higher oxygen pressures.

The inoculated animals were usually exposed to HBO at 2 to 3 ATA for 1 to 5 days or more by exposures of 2 to 4 hrs at intervals of 2 to 4 hrs. The exposure varied from one to six times daily and the total exposure time from 4 to 30 hrs.

The observation of infected animals after termination of the experiments lasted up to 6 months.

Results

(1) In the group of experimental animals inoculated intratesticularly and 5 days later exposed to HBO (2 ATA, for 2 hrs repeated four times daily at 2-hr intervals), clinical manifestations in the inoculated testicles, if not already present, were postponed for about 4 weeks. However, if clinical signs (beginning of swelling and inflammation) were established before the exposure to HBO (*i.e.* 5 days after inoculation), there was first an intensification of inflammation and a few days later a return to normal. As a rule about 30 days after the termination of hyperbaric oxygenation, the infected testicles became enlarged, swollen, and hard, and soon necrosed and ulcerated.

Darkground examination for *T. pallidum* in these testicles at the beginning of hyperbaric oxygenation, and particularly at the end of the course, generally showed first a reduction in the number of the organisms and usually later a complete absence. However, this absence of treponemes was transient, because in a few days (usually 8) the darkfield examination again became positive.

Regarding the serological findings in this group of experiments, there was no difference between the experimental and control animals which were not exposed to HBO in respect of the onset of positive VDRL tests, but in the HBO exposed animals the

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titre later usually became higher than in the control group at the same period of time after inoculation.

(2) The next group of experiments relate to intratesticular inoculation of rabbits which were exposed to HBO (4×4 hrs at 2 ATA with intervals of 2 hrs—total exposure time 16 hrs) 30 days after inoculation.

In this group the HBO produced no special effect on the exposed animals and no essential difference could be observed in the further course of the infection between the exposed and control animals. Exceptionally, shortly after exposure to HBO, a transient increased inflammation of the infected testicle occurred.

(3) In these experiments it was investigated whether the infection could be prevented or aborted when the exposure to HBO took place immediately after intravenous inoculation of the experimental animals.

The intravenous inoculation was carried out with 1 ml. of *Treponema pallidum* Nichols strain suspension (6×10^7).

The exposure to HBO was varied as follows:
(*a*) 4 hrs continuously at 3 ATA (total 4 hrs)
(*b*) 3 hrs at 2 ATA daily for 10 days (total 30 hrs)
(*c*) 4×4 hrs at intervals of 2 hrs (total 16 hrs).

In all the infected animals, regardless of the scheme of exposure to HBO, *T. pallidum*-positive skin lesions developed on average 3 to 4 weeks after inoculation, analogous to the control animals which were not exposed to HBO.

There was also no difference in regard to the onset of positive serology except that in animals exposed to HBO a higher titre developed at the beginning than in the control animals, but later in the course of the infection this difference disappeared.

(4) When the tintravenously inoculated animals were exposed to HBO (7×2 hrs at 3 ATA with intervals of 2 hrs; total 14 hrs) near the end of the incubation period (20 days after inoculation), the development of skin lesions was postponed for about 14 days (6 weeks after inoculation) in comparison to the non-exposed animals inoculated the same day with the same suspension of treponemes.

(5) When the skin lesions had already developed (Fig. 1), and the experimental animal was then exposed to HBO ($2\frac{1}{2}$ hrs at 2·5 ATA daily for 10 days—total 25 hrs) a well-marked diminution of the skin lesions after intravenous inoculation was seen and they usually disappeared after a few exposures (Fig. 2). This was in contrast to the animals not exposed to HBO, in which the skin lesions showed further progression (Fig. 3). However, the darkfield examination was usually positive, even though a distinct improvement in the clinical condition could be observed in the animals which were exposed to HBO.

(6) In latent syphilis no influence of HBO, either clinical or serological, could be seen during the time of observation (6 mths).

HISTOPATHOLOGY

The histological investigations were of skin lesions in experimental animals which were infected by different routes and exposed to hyperbaric oxygenation. The biopsies were taken before and at different times after exposure to HBO and compared with the histology of control animals which were inoculated in the same way and with the same suspension of *T. pallidum*, but not exposed to HBO.

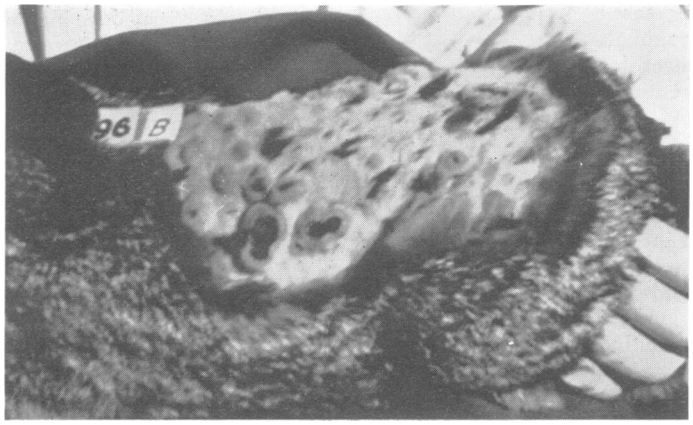

FIG. 1 *Skin lesions in a syphilitic rabbit inoculated intravenously before exposure to HBO*

(1) After intradermal inoculation, biopsies were taken from the skin lesions 15 days after inoculation and the animal then exposed to HBO (4 × 2 hrs daily at 2 ATA for 3 days–total exposure time 24 hrs).

The biopsies for histological examination in the exposed and control animals were taken after 8, 16, and 24 hrs, and on the 15th and 30th days.

(2) After intravenous inoculation, the experiment started 4 weeks later when skin lesions had already developed and the animals were then exposed to HBO (3 ATA 3 hrs daily for 2 days—total 6 hrs).

Biopsies for histological examination in the exposed and control animals were performed before, and on the first and second day immediately after exposure to HBO.

The histological preparations were stained with haematoxylin and eosin, Van Gieson, and various special stains for histochemical analysis which will be described in detail elsewhere.

HISTOPATHOLOGICAL FINDINGS

The histopathological findings before exposure to HBO were typical of early skin lesions in experimental rabbit syphilis, with developing infiltration located particularly around the vessels and composed mainly of lymphocytes, 'pseudo-eosinophil', and plasma cells (Fig. 4) and proliferation and swelling of the endothelial cells with thickening and invasion of the walls of blood vessels (Fig. 5) leading to partial or complete obliteration of the lumina resulting in foci of necrosis.

After exposure to HBO, even after 8 hrs exposure at 2 ATA, the histopathological picture had changed. Cellular infiltration was composed mostly of plasma cells (Fig. 6) and the proliferation and swelling of endothelial cells of capillaries and blood vessels were markedly reduced so that their lumina became clear (Fig. 7) and the walls less thickened and almost normal in appearance. The same could be achieved after 3 hrs' exposure to HBO at 3 ATA.

Especially after a second exposure of 3 hrs at 3 ATA or 16 to 24 hrs after exposure at 2 ATA, the polymorphous cell infiltration changed and plasma cells predominated; also cells in division appeared in the infiltrate which seemed to belong to the same group of mononuclear cells (Fig. 8, overleaf).

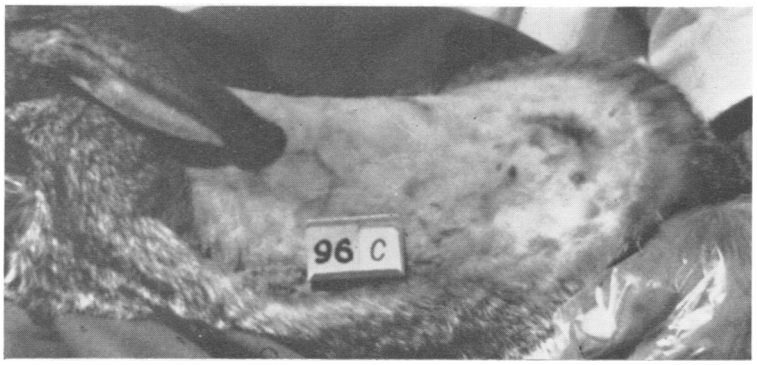

FIG. 2 *Same rabbit as in Fig* 1, *but after exposure to HBO* (3 *weeks later*)

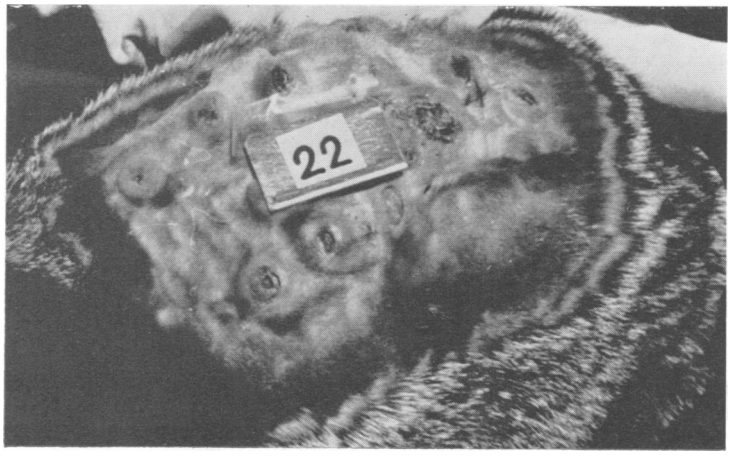

FIG. 3 *Skin lesions in a control rabbit not exposed to HBO*

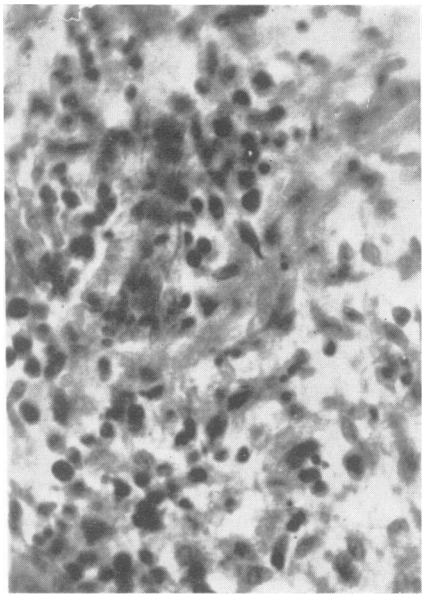

FIG. 4 *Biopsy of a skin lesion from a syphilitic rabbit before exposure to HBO. Diffuse polymorphous infiltration.* ×400

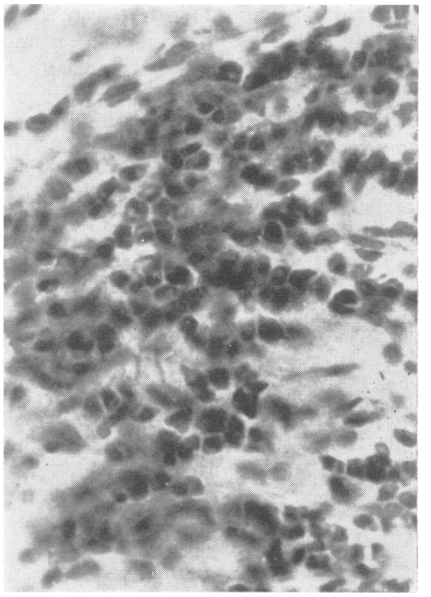

FIG. 6 *Cellular infiltration composed mostly of plasma cells in a biopsy of a skin lesion in a syphilitic rabbit exposed to HBO twice for 4 hrs at 2 ATA.* ×400

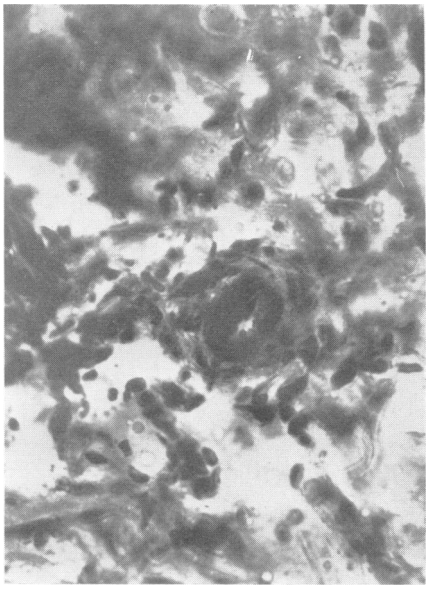

FIG. 5 *Biopsy of a skin lesion from a syphilitic rabbit before exposure to HBO. Typical thickening of blood vessels.* ×400

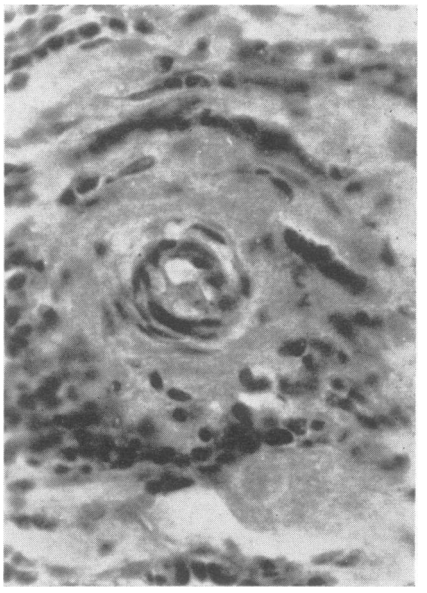

FIG. 7 *Biopsy of a skin lesion from a syphilitic rabbit after exposure to HBO four times for 2 hrs at 2 ATA. Normalization of pathological vascular changes.*

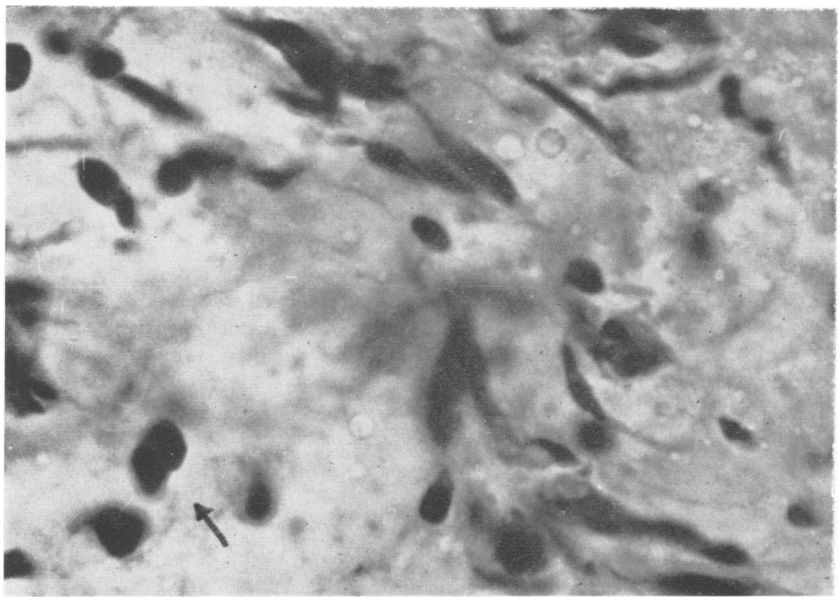

FIG. 8 *Biopsy of a skin lesion after exposure of a syphilitic rabbit to HBO for 3 hrs at 3 ATA. Division of mononuclear cell.* ×600

In areas where necrosis had developed, an accelerated process of repair and elimination of the damaged tissue indicated by the presence of polynuclear cells and macrophages was established.

The histopathology of biopsies taken from control animals unexposed to HBO showed after the same time the picture of progressive inflammation and destruction. The difference was even more evident 15 and 30 days respectively after exposure to HBO. The necrotic foci at that time in the animals exposed to HBO displayed in histological preparations obvious signs of advanced and intensive reparative processes with production of newly formed connective tissue (Fig. 9), whereas in the control animals the destruction and inflammation (Fig. 10, overleaf) were still progressing or the reparative process was just beginning.

It could also be demonstrated histochemically

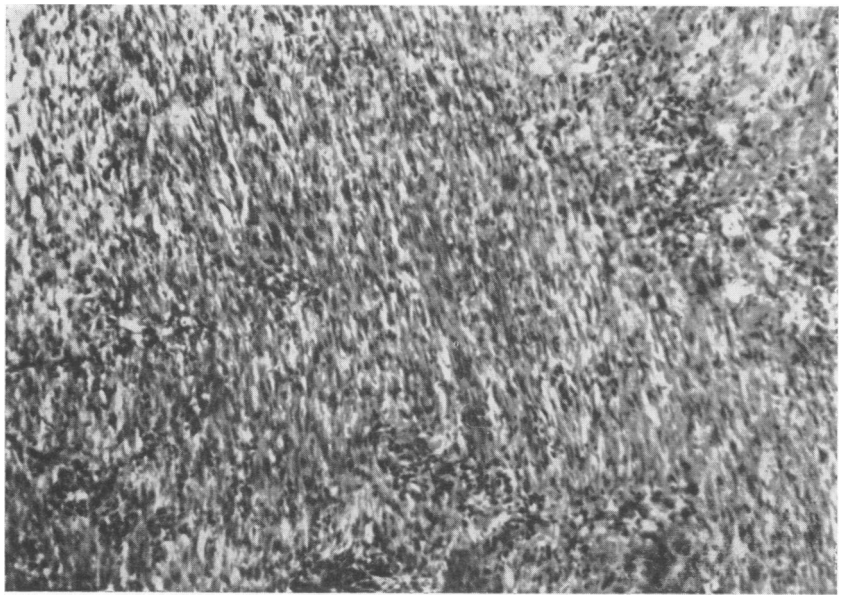

FIG. 9 *Biopsy of a skin lesion 15 days after exposure to HBO. Intensive reparative activity with production of newly formed connective tissue.* ×200

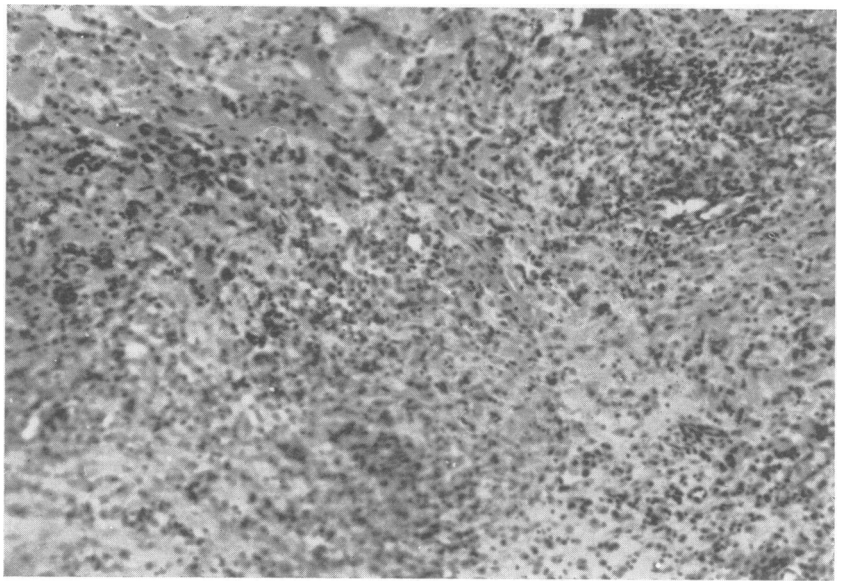

FIG. 10 *Biopsy of a skin lesion from a control animal not exposed to HBO. Inflammation and progressive tissue destruction.* ×200

that the mucoid metachromatic material, which is abundant in the proliferative stage of skin lesions in experimental rabbit syphilis (Turner and Hollander 1957), became markedly reduced after exposure to HBO.

Discussion

Although the requirement for anaerobiasis of *T. pallidum* is generally accepted, doubts on this belief and on the anti-treponemicidal action of oxygen *per se* have been expressed (Turner and Hollander, 1957).

It is obvious, from our present knowledge, that *T. pallidum* requires *in vitro* in survival media an exceptionally low oxidation-reduction potential (*T. pallidum* cannot be cultivated *in vitro*), but there is very limited and mostly unverified evidence available regarding the need for anaerobiasis of *T. pallidum in vivo* (proliferation of *T. pallidum* in old granulating wounds, predilection for syphilitic lesions at sites of trauma, numerous *T. pallidum* in the macerated syphilitic foetus, etc.), and regarding the influence of oxygenation and the requirements needed (physical or nutritional) for the growth of *T. pallidum* in tissue (Hardy, 1969); Turner and Hollander, 1957; Chesney, 1928; Willcox and Guthe, 1966).

Our experiments on the effect of HBO under various conditions on rabbit syphilis, demonstrated that the toxic influence of oxygen on *T. pallidum in vivo* is not lethal and cannot prevent or abort the infection if the experimental rabbit is exposed in the incubation period to pure oxygen at a tension of 2 to 3 ATA intermittently for up to 30 hrs. A longer exposure time and increased tension was in general lethal to the animal itself.

When the infected rabbits were exposed to HBO immediately or shortly after infection but before clinical lesions had developed, the differences between the exposed and control animals were found to be small, but when the animal was exposed to HBO in the exponential stage of infection (Collart, Franceschini, and Durel, 1971), there was an evident stimulatory effect on the defence mechanism and the repair of pathological changes caused by the treponeme.

In contrast, our investigations have shown that suspensions of motile *T. pallidum* in 50 per cent. rabbit serum exposed *in vitro* to HBO at 2 and 3 ATA for 20 and 15 min. respectively completely lost their motility.

Clearly there is an essential difference between the oxygen sensitivity of *T. pallidum in vitro* and in the infected animal, but its mode of action is still not well understood.

It is known (Meijne, 1970) that by exposure to HBO at 3 ATA a rise of about 25 per cent. in O_2 content is achieved in the arterial blood, but there is no evidence that an increased pO_2 alters the oxygen consumption in tissue cells adequately supplied with oxygen unless oxygen toxicity occurs (Horne, 1965; Telfer and Jennett, 1965).

Nevertheless, our experiments demonstrate that HBO has a definite effect in syphilitic rabbits. At first *T. pallidum* became less numerous in active lesions after oxygenation and may transiently

disappear from darkfield preparations. The explanation of this phenomenon can be only speculated upon. It might be supposed with considerable probability that hyperbaric oxygen produces unfavourable conditions for the existence of *T. pallidum* in spiral form, but it retains its virulence and germinative ability in some other more resistant form, which after cessation of the harmful influence of hyperbaric oxygenation, again develops an abundant growth of spiral forms. Such ability of treponemes to produce non-spiral forms under adverse circumstances under the action of penicillin has recently been described in electron microscopic studies (Ovčinnikov and Delektorskij, 1971), and it may be assumed that HBO is a stressful factor having a similar influence on treponemes *in vivo*.

At present it cannot be established how far HBO affects *T. pallidum* directly and how far it affects the host defence mechanism which is evidently stimulated by HBO as proved by histopathological findings in the skin lesions. It appears that the beneficial effect of HBO on the clinical course of skin lesions seems to be based on the stimulation of cellular and tissue repair activity (increased phagocytosis, reduced inflammation, elimination and repair of damaged tissue, and regeneration) rather than on a direct treponemicidal effect *in vivo*.

Summary

An investigation was conducted on the effect of hyperbaric oxygenation (HBO) in experimental rabbit syphilis, assuming that the exposure of infected animals to a high tension of pure oxygen might have a toxic effect similar to that on *Treponema pallidum in vitro* (which is strictly anaerobic).

The experiments were carried out (including controls) on 64 rabbits, which were inoculated testicularly (36), intravenously (18), or subcutaneously (10) with the pathogenic Nichols strain.

The infected animals were exposed in various tests to HBO, usually at 2 to 3 atmospheres absolute (ATA) for 2 to 4 hrs at intervals of from 2 to 4 hrs. The daily exposures varied from one to six and the, total exposure time from 4 to 30 hrs.

Rabbits inoculated intratesticularly were exposed to HBO 5 days after inoculation. If clinical manifestations were not already present their onset was postponed (for about 4 weeks), but if clinical lesions had already developed, a transient return to normal could be achieved. In darkfield examinations treponemes became less numerous, and a complete but transient absence of treponemes usually resulted.

HBO could not prevent or abort the infection in animals inoculated intravenously when given immediately after inoculation, but when applied later, near the end of the inoculation period, it postponed the appearance of skin lesions.

When skin lesions had already developed, HBO had a distinct stimulative effect on regenerative activity and normalization of the lesions; this could be demonstrated clinically and histopathologically.

Little influence of HBO on the serological tests in the exposed animals could be detected.

The toxic influence of HBO on *T. pallidum in vivo* under the experimental conditions in these investigations did not prove to be lethal, although a beneficial effect on the lesions in the syphilitic rabbit could be demonstrated. This effect seemed to be caused by stimulation of the host defence mechanism rather than by a directly treponemicidal action.

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Effet de l'hydrogène hyperbare (OHB) dans la syphilis expérimentale du lapin

SOMMAIRE

Une recherche a été effectuée sur l'effet de l'oxygénation hyperbare (OHB) dans la syphilis expérimentale du lapin avec l'idée que la soumission d'animaux infectés à une haute tension d'oxygène pur pourait avoir une action toxique *in vitro* sur *Treponema pallidum* (qui est anaérobie strict).

Les essais portèrent (témoins compris) sur 64 lapins inoculés par voie testiculaire (36), intraveineuse (18) ou sous-cutanée (10) avec la souche Nichols pathogène.

Les animaux infectés furent exposés à l'OHB et soumis à des tests variés; l'oxygène fut généralement administré à 2 ou 3 atmosphères (en absolu) (ATA) pendant 2 à 4 heures, à intervalles de 2 à 4 heures. Les expositions quotidiennes allèrent de 1 à 6 et le temps total d'exposition de 4 à 30 heures.

Les lapins inoculés par voie testiculaire furent exposés à l'OHB cinq jours après l'inoculation. Si les manifestations cliniques n'étaient pas déjà apparu, celles-ci furent retardées (d'environ quatre semaines) mais si une lésion clinique s'était déjà developpée, un retour passager à la normale put être obtenu. Au fond noir, le nombre des tréponèmes diminua pour arriver généralement à une absence complète, mais transitoire, de tréponèmes.

L'OHB ne peut pas prévenir ou faire avorter l'infection chez les animaux inoculés par voie intraveineuse lorsqu'il est administré immédiatement après l'inoculation, mais, administré plus tard, vers la fin de la période d'incubation, il retarde l'apparition des lésions cutanées.

Si les lésions cutanées étaient déjà apparues, l'OHB eut une action stimulante nette sur l'activité régénératrice et la normalisation des lésions, comme ceci fut démontré cliniquement et histo-pathologiquement.

On ne put trouver aucune influence de l'OHB sur la sérologie des animaux qui y furent soumis.

L'influence toxique de l'OHB sur les tréponèmes *in vivo*, dans les conditions expérimentales des présentes recherches n'eut pas d'action létale, bien que l'on put constater un effet benéfique sur les lésions de la syphilis du lapin. Cet effet a semblé être plus dû au mécanisme de défense de l'hôte qu'à une action tréponémicide directe.